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- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ASSAY METHOD FOR DETECTING FUNGAL INFECTION OF SOIL OR VEGETABLES

(57) Abstract: The invention provides an assay method for detecting fungal infection of soil or vegetables by pathogenic fungal species, in particular *M. acerina*, *F. carotae* and *Pythium* species, said method comprising: obtaining a sample of soil or vegetable; treating said sample to lyse fungal cells therein; using an oligonucleotide primer pair, effecting a polymerase chain reaction on DNA released by lysis of the fungal cells; and detecting DNA fragments generated by said polymerase chain reaction; wherein said primer pair comprises an 18- to 24-mer having the ability to hybridize to one of the oligonucleotide sequences of formulae (Ia), (Ib), (IIa), (IIb), (IIIa), (IIIb), (IVa), (IVb), (Va), (Vb), (VIa), (VIb), (VIIa), (VIIb), (VIIIa), (VIIIb), (IXa), (IXb), (Xa), (Xb), (XIa), (XIb), (XIIa), (XIIb), (XIIIa), (XIIIb), (XIVa) and (XIVb).

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 03/04712

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, MEDLINE, PAJ, WPI Data, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KAGEYAMA K ET AL: "DETECTION OF PYTHIUM ULTIMUM USING POLYMERASE CHAIN REACTION WITH SPECIES-SPECIFIC PRIMERS" PLANT DISEASE, AMERICAN PHYTOPATHOLOGICAL SOCIETY, ST. PAUL, MN, US, vol. 81, October 1997 (1997-10), pages 1155-1160, XP009024662 ISSN: 0191-2917 page 1155, column 3, lines 23-48 page 1156, column 1, line 4 - column 3, line 3 table 1 figure 1  ----- -/--	1,2,4, 6-8, 10-15,17

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

### \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 03/04712

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MATSUMOTO CHIEKO ET AL: "Phylogenetic relationships of Pythium species based on ITS and 5.8S sequences of the ribosomal DNA" MYCOSCIENCE, vol. 40, no. 4, 15 August 1999 (1999-08-15), pages 321-331, XP001180153 ISSN: 1340-3540 page 329, column 2, lines 37-39 figure 1	1,2,4, 6-8, 10-15,17
Y	LEVESQUE C A ET AL: "The use of DNA arrays for direct detection of oomycetes from roots and soils" CANADIAN JOURNAL OF PLANT PATHOLOGY, vol. 22, no. 2, June 2000 (2000-06), page 188, XP009028449 Annual Meeting of the Canadian Phytopathological Society, 2000;Victoria, British Colombia, Canada ISSN: 0706-0661 abstract	1,2,4, 6-8, 10-15,17
Y	QUAIL A ET AL: "Pythium identification by hybridization to a DNA array" CANADIAN JOURNAL OF PLANT PATHOLOGY, vol. 22, no. 2, June 2000 (2000-06), page 191, XP009028447 Annual Meeting of the Canadian Phytopathological Society, 2000;Victoria, British Colombia, Canada ISSN: 0706-0661 abstract	1,2,4, 6-8, 10-15,17
Y	WANG P H ET AL: "Specific detection of Pythium aphanidermatum from hydroponic nutrient solution by booster PCR with DNA primers developed from mitochondrial DNA." PHYTOPARASITICA, vol. 30, no. 5, October 2002 (2002-10), pages 473-485, XP009027978 ISSN: 0334-2123 the whole document	1,2,4, 6-8, 10-15,17

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 03/04712

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LEVESQUE C ANDRE ET AL: "Identification of some oomycetes by reverse dot blot hybridization" PHYTOPATHOLOGY, vol. 88, no. 3, March 1998 (1998-03), pages 213-222, XP001180427 ISSN: 0031-949X table 1 page 217, column 1, line 9 - column 2, line 3 figure 2 page 221, column 1	1,2,4, 6-8, 10-15,17
Y,P	KAGEYAMA KOJI ET AL: "Refined PCR protocol for detection of plant pathogens in soil." JOURNAL OF GENERAL PLANT PATHOLOGY, vol. 69, no. 3, June 2003 (2003-06), pages 153-160, XP002275151 ISSN: 1345-2630 (ISSN print) page 155, column 1, line 20 - column 2, line 9 page 154, column 1, line 47 - column 2, line 15	1,2,4, 6-8, 10-15,17
Y,P	BARASUBIYE T ET AL: "Molecular identification and detection of root rot pathogens in soybean." PHYTOPATHOLOGY, vol. 93, no. 6 Supplement, June 2003 (2003-06), page S6, XP002275152 Annual Meeting of the American Phytopathological Society;Charlotte, North Carolina, USA; August 09-13, 2003 ISSN: 0031-949X (ISSN print) abstract	6,11,12
Y,P	WANG P H ET AL: "Use of polymerase chain reaction to detect the soft rot pathogen, <i>Pythium myriotylum</i> , in infected ginger rhizomes." LETTERS IN APPLIED MICROBIOLOGY, vol. 36, no. 2, 2003, pages 116-120, XP001180354 ISSN: 0266-8254 page 117, column 2, lines 7-10 figures 1-3 table 1	1,2,4, 6-8, 10-15,17
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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 03/04712

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	<p>WANG P H ET AL: "Detection of the low-germination-rate resting oospores of <i>Pythium myriotylum</i> from soil by PCR." LETTERS IN APPLIED MICROBIOLOGY. ENGLAND 2003, vol. 36, no. 3, 2003, pages 157-161, XP001180356 ISSN: 0266-8254 figures 1-4</p>	1,2,4, 6-8, 10-15,17
Y,P	<p>WANG P H ET AL: "Species-specific PCR primers for <i>Pythium</i> developed from ribosomal ITS1 region." LETTERS IN APPLIED MICROBIOLOGY. ENGLAND 2003, vol. 37, no. 2, 2003, pages 127-132, XP001180355 ISSN: 0266-8254 tables 1-3</p>	1,2,4, 6-8, 10-15,17
T	<p>WANG P H ET AL: "Erratum: Species-specific PCR primers for <i>Pythium</i> developed from ribosomal ITS1 region (Letters in Applied Microbiology (2003) 37 (127-132))" LETTERS IN APPLIED MICROBIOLOGY 2004 UNITED KINGDOM, vol. 38, no. 1, 2004, page 78, XP001180357 ISSN: 0266-8254 the whole document</p>	1,2,4, 6-8, 10-15,17

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/GB 03/04712

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1 (partially), 2 & 4 (completely), 6 & 7 (partially), 8 (completely)  
10-14 (part.), 15 (comp.), 17 (part.)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1 (partially), 2 and 4 (completely), 6 and 7 (partially), 8 (completely), 10-14 (partially), 15 (completely), 17 (partially)

An 18- to 24-mer oligonucleotide primer hybridizable to an oligonucleotide sequence selected from the formulae Ia, Ib, IIa, IIb, IIIa, IIIb, IVa, IVb, Va, Vb, VIa, VIb, VIIa, VIIb, VIIa, VIIb, IXa, IXb, Xa, Xb and its use in a composition, in a kit, immobilized on a substrate or in an assay method for detecting fungal infection of soil or vegetables by pathogenic fungal species, in particular *Pythium* species.

- 1.1. claims: 1 (partially), 2 and 4 (completely), 6 and 7 (partially), 8 (completely), 10-14 (partially), 15 (completely), 17 (completely)

An assay method for detecting fungal infection of soil or vegetables by pathogenic fungal species, in particular *Pythium* species by PCR wherein the primer pair comprises an 18- to 24-mer having the ability to hybridize to one of the oligonucleotide sequences of formulae Ia (SEQ ID N° 1), Ib (SEQ ID N° 2), IIa (SEQ ID N° 3), IIb (SEQ ID N° 4), IIIa (SEQ ID N° 5), IIIb (SEQ ID N° 6), IVa (SEQ ID N° 7), IVb (SEQ ID N° 8), Va (SEQ ID N° 9), Vb (SEQ ID N° 10), VIa (SEQ ID N° 11), VIb (SEQ ID N° 12), VIIa (SEQ ID N° 13), VIIb (SEQ ID N° 14), VIIa (SEQ ID N° 15), VIIb (SEQ ID N° 16), IXa (SEQ ID N° 17), IXb (SEQ ID N° 18), Xa (SEQ ID N° 19), Xb (SEQ ID N° 20), or wherein the primer is immobilized on a substrate.

An 18- to 24-mer oligonucleotide primer or a primer composition comprising a 18- to 24-mer oligonucleotide primer hybridizable to an oligonucleotide sequence selected from the formulae Ia, Ib, IIa, IIb, IIIa, IIIb, IVa, IVb, Va, Vb, VIa, VIb, VIIa, VIIb, VIIa, VIIb, IXa, IXb, Xa, Xb.

A substrate having immobilized thereon at least one 18- to 24-mer oligonucleotide primer hybridizable to an oligonucleotide sequence selected from the formulae Ia, Ib, IIa, IIb, IIIa, IIIb, IVa, IVb, Va, Vb, VIa, VIb, VIIa, VIIb, VIIa, VIIb, IXa, IXb, Xa, Xb.

A kit for the performance of the array method.

- 1.2. claims: 1 (partially), 3 and 5 (completely), 6 and 7 (partially), 9 (completely), 10-14 (partially), 16 (completely), 17 (completely)

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

An assay method for detecting fungal infection of soil or vegetables by pathogenic fungal species, in particular *M. acerina* and *F. carotae* by PCR wherein the primer pair comprises an 18- to 24-mer having the ability to hybridize to one of the oligonucleotide sequences of formulae XIa (SEQ ID N° 21), XIb (SEQ ID N° 22), XIIa (SEQ ID N° 23), XIIb (SEQ ID N° 24), XIIIa (SEQ ID N° 25), XIIIb (SEQ ID N° 26), XIVa (SEQ ID N° 27), XIVb (SEQ ID N° 28) or wherein the primer is immobilized on a substrate.

An 18- to 24-mer oligonucleotide primer or a primer composition comprising a 18- to 24-mer oligonucleotide primer hybridizable to an oligonucleotide sequence selected from the formulae XIa (SEQ ID N° 21), XIb (SEQ ID N° 22), XIIa (SEQ ID N° 23), XIIb (SEQ ID N° 24), XIIIa (SEQ ID N° 25), XIIIb (SEQ ID N° 26), XIVa (SEQ ID N° 27), XIVb (SEQ ID N° 28).

A substrate having immobilized thereon at least one 18- to 24-mer oligonucleotide primer hybridizable to an oligonucleotide sequence selected from the formulae XIa (SEQ ID N° 21), XIb (SEQ ID N° 22), XIIa (SEQ ID N° 23), XIIb (SEQ ID N° 24), XIIIa (SEQ ID N° 25), XIIIb (SEQ ID N° 26), XIVa (SEQ ID N° 27), XIVb (SEQ ID N° 28).

A kit for the performance of the array method.

2. claims: 1 (partially), 3 and 5 (completely), 6 and 7 (partially), 9 (completely), 10-14 (partially), 16 (completely), 17 (partially)

An 18- to 24-mer oligonucleotide primer hybridizable to an oligonucleotide sequence selected from the formulae XIa (SEQ ID N° 21), XIb (SEQ ID N° 22), XIIa (SEQ ID N° 23), XIIb (SEQ ID N° 24), XIIIa (SEQ ID N° 25), XIIIb (SEQ ID N° 26), XIVa (SEQ ID N° 27), XIVb (SEQ ID N° 28) and its use in a composition, in a kit, immobilized on a substrate or in an assay method for detecting fungal infection of soil or vegetables by pathogenic fungal species, in particular *M. acerina* and *F. carotae*.

3. claims: 18-21

A process and a kit for the extraction of nucleic acid.